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## ORIGINAL PAPER

# Description of *Scottnema lindsayae* Timm, 1971 (Rhabditida: Cephalobidae) from Taylor Valley, Antarctica and its phylogenetic relationship

Sven Boström · Oleksandr Holovachov · Steven A. Nadler

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**Abstract** The endemic Antarctic nematode *Scottnema lindsayae* is described from specimens collected in Taylor Valley, McMurdo Dry Valleys, Victoria Land. The recently collected material is compared with the original description and other subsequent descriptions of the species. A more complete scanning electron microscopy (SEM) study of the species is presented. The phylogenetic position of *S. lindsayae* is inferred using a secondary structure-based alignment of a partial sequence of nuclear Large Subunit (LSU) ribosomal DNA. Phylogenetic trees were inferred using base-paired substitution models implemented in PHASE 2 software and Bayesian inference, and show *S. lindsayae* as the sister group to *Stegelletina* taxa.

**Keywords** Antarctica · Cephalobidae · Description · Morphology · Nematoda · Phylogeny · *Scottnema* · SEM · Taxonomy · Taylor Valley

## Introduction

The endemic nematode *Scottnema lindsayae* Timm, 1971 is one of only a few soil nematode species found in the

extreme environmental conditions of the Antarctic Dry Valleys, South Victoria Land (77–78°S). It has also been reported from the Syowa Station in East Antarctica (~71°S), on the other side of the continent, by Shishida and Ohyama (1986), from the Antarctic Peninsula (~62°S) by Mouratov et al. (2001), and recently as far south as the Transantarctic Mountains (~83°S) by Adams et al. (2007). Its life history, spatial distribution (horizontal and vertical), dispersal and different aspects of its ecology have been investigated in several studies (e.g. Overhoff et al. 1993; Powers et al. 1995; Treonis et al. 1999; Courtright et al. 2001; Porazinska et al. 2002a, b; Moorhead et al. 2002, 2003; Gooseff et al. 2003; Weicht and Moorhead 2004; Nkem et al. 2006a, b; Adams et al. 2007; Ayres et al. 2008, 2010; Barrett et al. 2008; Simmons et al. 2009). The species was originally described by Timm (1971) from a locality near La Croix Glacier, Taylor Valley in Victoria Land. He also reported presence of the species at Ross Island and several other localities in Taylor Valley. Other populations from Ross Island and from many localities in Victoria Land have subsequently been characterized by morphological (Andrássy 1998; Vinciguerra 1994) and molecular methods (Courtright et al. 2000). Courtright et al. (2000) did not observe any fixed differences in nuclear rRNA sequences between populations, but found variation in mitochondrial DNA sequences defining several haplotypes. They concluded that: “The nuclear and mitochondrial variation suggests populations of *S. lindsayae* represent a single polymorphic species with some restriction of gene flow between geographic populations.”

*Scottnema lindsayae* is a microbivorous nematode feeding on bacteria, yeast and algal cells. It has been found in several types of habitats: sand or mineral soils with mixed fractions of sand, gravel and stones; moss; green algal mats (e.g. *Prasiola*); and lake bottom detritus. It has a

S. Boström (✉)  
Department of Invertebrate Zoology, Swedish Museum  
of Natural History, Box 50007, 104 05 Stockholm, Sweden  
e-mail: [sven.bostrom@nrm.se](mailto:sven.bostrom@nrm.se)

O. Holovachov  
Department of Nematology, University of California-Riverside,  
Riverside, CA 92521, USA

S. A. Nadler  
Department of Nematology, University of California-Davis,  
Davis, CA 95616, USA

comparatively long life cycle (218 days at 10°C), indicating that more than one austral summer is required for its completion, and a low fecundity in comparison with many other genera of Cephalobidae (Overhoff et al. 1993). During periods of extreme temperature and/or desiccation, the nematodes will survive by anhydrobiosis entering a cryptobiotic stage (Treonis et al. 2000; Weicht and Moorhead 2004; Treonis and Wall 2005).

In this paper, a population of *S. lindsayae* from Taylor Valley (77°S) is described by using light (LM) and scanning electron microscopy (SEM) and compared with the original and subsequent descriptions of the species. The phylogenetic position of *S. lindsayae* is inferred from partial Large Subunit (LSU) nuclear ribosomal DNA sequences.

## Methods

Samples were collected by Olle Karlsson on 19 January 2009 in Taylor Valley, Victoria Land, close to Lake Hoare and Lake Chad. The area is characterized by a moraine with sand, gravel and larger blocks of stone, with permafrost beginning at 35–50 cm depth. The samples were collected in the area 77°37.6'S, 162°52.1'E to 77°38.6'S, 162°44.2'E and generally consisted of dry or somewhat moist mixed fractions of sand, coarse sand and gravel.

Specimens were recovered from soil samples using a modified Baermann funnel extraction technique, where the individual samples weighed between 11 and 24 gram dry weight (gdw). The nematodes were relaxed by gentle heat and fixed in cold TAF for morphological studies and in DESS (Yoder et al. 2006) for molecular analysis. For LM, nematodes were transferred to pure glycerine by a slow evaporation method and mounted on permanent slides in glycerine with paraffin wax as support for the coverslip. Measurements are given for individual specimens or a range of specimens, as indicated. For SEM studies, some specimens were postfixed in 1% osmium tetroxide (OsO<sub>4</sub>) and transferred to pure acetone through an acetone/distilled water series. Specimens were critical point dried in liquid CO<sub>2</sub>, mounted on stubs, gold-plated under vacuum to a thickness of 200 Å in a sputter coater, and examined in a Hitachi S-4300 SEM at an accelerating voltage of 10 kV. DESS-preserved specimens were used for DNA extraction and PCR as described in Holovachov et al. (2009b) and deposited in GenBank (Accession #HM439773 and HM439774). Eight individual nematodes from each sample were processed in bulk for PCR and sequencing. Sequences generated from specimens from two samples showed no evidence of polymorphism and were identical to each other. Phylogenetic analyses were

performed in a framework of selected sequence data (see Table 1) available in public databases for Cephalobioidea and selected representatives from the order Rhabditida *sensu* De Ley and Blaxter (2004), using a secondary structure-based alignment created as described in Holovachov et al. (2009b) with 4SALE software (Seibel et al. 2006). Furthermore, eight new cephalobid sequences were added to the dataset, from our most recent sampling in Sweden and southern California desert habitats (Table 2). These species were also preserved in DESS and processed in the same way for PCR and sequencing as *Scottnema lindsayae*. The resulting alignment was analysed with Bayesian phylogenetic inference using the *mcmcphase* program in the PHASE package (Gowri-Shankar and Jow 2006; Version 2.0). For this analysis, the dataset was partitioned into “stems” (paired sites) and “loops” (non-paired sites) to account for the potential phylogenetic importance of compensatory substitutions. The REV nucleotide substitution model (Tavare 1986) was used for non-paired sites, whereas RNA7A (Higgs 2000), RNA7D (Tillier and Collins 1998) and RNA16A (Gowri-Shankar and Jow 2006) nucleotide substitution models were used for paired sites in three independent analyses. For each model combination, we performed two independent runs; and for each run, chains were allowed to burn in for 500,000 generations, followed by 5 million generations (total 5.5 million generations) during which tree topologies, branch length and model parameters were sampled every 200 generations.

## Results

Of the 11 samples collected, two were devoid of metazoans. Nine samples contained *S. lindsayae* and in two of them single specimens of *Eudorylaimus* were also found. One sample contained a single tardigrade of the genus *Macrobiotus*. In the samples where *Scottnema* was found, the mean number of specimens varied between 0.05 and 10.2 per gram dry weight (gdw) of the extracted sample. Samples with few nematodes contained only females and juveniles, whilst those with higher densities appeared to be active growing populations with females, males and juveniles. The abundance of nematodes was consistent with that recorded by Courtright et al. (2001) from Taylor Valley. They found 2.1–8.6 specimens per gdw when using a sugar centrifugation extraction method with individual samples of 100 g soil.

In many specimens, a string of mineral material that appeared to be stuck together was observed in the intestine, from just behind the cardia almost to rectum. Timm (1971) also recorded that the intestine was filled with fine detritus.

**Table 1** List of nematode LSU ribosomal RNA gene sequences (partial) obtained from GenBank and used in this study

Accession number	Genus/species and strain where applicable	References
GU062821	<i>Geraldus</i> sp.	Holovachov et al. (2009a)
DQ145639	<i>Macrolaimus</i> sp. SAN2005	Nadler et al. (2006)
DQ145636	<i>Fescia grossa</i>	Nadler et al. (2006)
DQ145620	<i>Acrobeles complexus</i>	Nadler et al. (2006)
DQ145621	<i>Acrobeles maeneeneus</i>	Nadler et al. (2006)
DQ145622	<i>Acrobeles singulus</i>	Nadler et al. (2006)
DQ145623	<i>Acrobeles</i> sp. JB132	Nadler et al. (2006)
DQ145625	<i>Acrobeloides bodenheimeri</i>	Nadler et al. (2006)
DQ903104	<i>Acrobeloides buetschlii</i> SAN002	Smythe and Nadler (2007)
DQ903081	<i>Acrobeloides buetschlii</i> DWF1107	Smythe and Nadler (2007)
DQ145624	<i>Acrobeloides ellesmerensis</i>	Nadler et al. (2006)
EF417138	<i>Acrobeloides maximus</i>	Sonnenberg et al. (2007)
DQ903078	<i>Acrobeloides maximus</i> DF5048	Smythe and Nadler (2007)
EU195987	<i>Acrobeloides maximus</i> DF5048	Kiontke et al. (2007)
EF417139	<i>Acrobeloides nanus</i>	Sonnenberg et al. (2007)
DQ903076	<i>Acrobeloides nanus</i> BSS4	Smythe and Nadler (2007)
DQ903103	<i>Acrobeloides nanus</i> PS1959	Smythe and Nadler (2007)
DQ903075	<i>Acrobeloides nanus</i> BSS3	Smythe and Nadler (2007)
DQ903083	<i>Acrobeloides thornei</i> DWF1109	Smythe and Nadler (2007)
DQ903087	<i>Acrobeloides uberrinus</i> JB27	Smythe and Nadler (2007)
DQ145626	<i>Acromoldavicus mojavicus</i>	Smythe and Nadler (2007)
EU253570	<i>Cephalobus cubaensis</i> PS1197	Kiontke et al. (2007)
DQ903077	<i>Cephalobus persegnis</i> BSS5	Smythe and Nadler (2007)
DQ145629	<i>Cervidellus alutus</i>	Nadler et al. (2006)
AF331911	<i>Cervidellus alutus</i> PDL004	Stock et al. (2001)
DQ145630	<i>Cervidellus doorsselaeri</i>	Nadler et al. (2006)
DQ145631	<i>Cervidellus nefasiensis</i>	Nadler et al. (2006)
DQ145632	<i>Cervidellus</i> sp. JB138	Nadler et al. (2006)
GU062819	<i>Chiloplacus demani</i>	Holovachov et al. (2009a)
DQ145634	<i>Chiloplacus</i> sp. JB81	Nadler et al. (2006)
GU062820	<i>Deficephalobus desenderi</i>	Holovachov et al. (2009a)
DQ145635	<i>Eucephalobus</i> sp. JB55	Nadler et al. (2006)
DQ145638	<i>Heterocephalobellus</i> sp. JB8	Nadler et al. (2006)

**Table 1** continued

Accession number	Genus/species and strain where applicable	References
DQ145640	<i>Macrolaimellus</i> sp. SAN2005	Nadler et al. (2006)
DQ145642	<i>Metacrobeles amblyurus</i>	Nadler et al. (2006)
DQ145645	<i>Nothacrobeles borregi</i>	Nadler et al. (2006)
DQ145644	<i>Nothacrobeles spatulatus</i>	Nadler et al. (2006)
DQ145646	<i>Nothacrobeles triniglarus</i>	Nadler et al. (2006)
FJ744540	<i>Penjatinema novaezeelandiae</i>	Holovachov et al. (2009b)
HM060685	<i>Placodira lobata</i>	Holovachov et al. (2010)
AF143368	<i>Pseudacrobeles variabilis</i>	De Ley et al. (1999)
DQ145654	<i>Pseudacrobeles</i> sp. JB85	Nadler et al. (2006)
DQ145653	<i>Pseudacrobeles</i> sp. JB56	Nadler et al. (2006)
DQ145656	<i>Stegelleta</i> sp. JB75	Nadler et al. (2006)
AY027533	<i>Stegelletina similis</i>	Baldwin et al. (2001)
DQ145657	<i>Stegelletina</i> sp. SAN2005	Nadler et al. (2006)
DQ145659	<i>Stegelletina</i> sp. JB139	Nadler et al. (2006)
DQ145658	<i>Stegelletina</i> sp. JB64	Nadler et al. (2006)
GU062818	<i>Tricirronema trifilum</i>	Holovachov et al. (2009a)
DQ145662	<i>Zeldia punctata</i>	Nadler et al. (2006)
EU195988	<i>Zeldia punctata</i> PDL3	Kiontke et al. (2007)
DQ145663	<i>Zeldia spannata</i> JB140	Nadler et al. (2006)

*Scottinema lindsayae* Timm, 1971 (Figs. 1, 2, 3, 4).

## Measurements

See Table 3.

## Description

### Adult

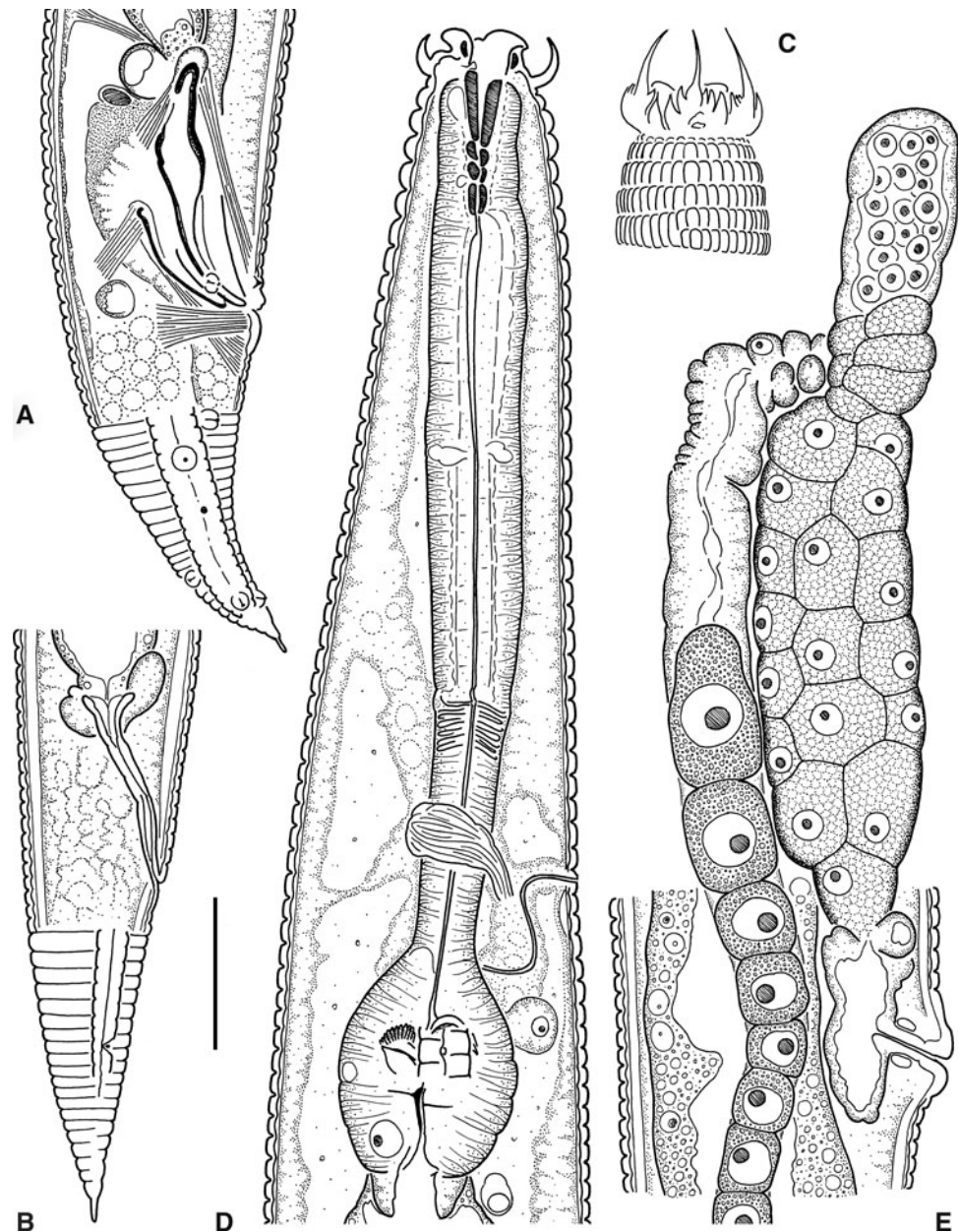
Body straight to slightly arcuate ventrad in females, ventrally curved posteriorly in males. Cuticle strongly annulated, annuli 1.9–2.8  $\mu\text{m}$  wide at midbody, 3.2–3.8  $\mu\text{m}$  anteriorly. Fine longitudinal striae give the cuticle a tiled appearance. Lateral field consisting of two alae, i.e. three incisures of which the outer two are crenate; occupying about 15–20% of body diameter. Lip region with six labial and four cephalic papillae. Amphid openings rounded, at base of lateral lips. Lip region strongly offset, consisting of six asymmetrical, flattened lips arranged in three pairs: one dorsal and two subventrals. Pairs of lips separated by primary axils with two triangular and elongate-pointed guarding processes originating from the base of the lip region; secondary axils demarcated by incisures separating the two lips of each pair. Cephalic probolae with four tines:



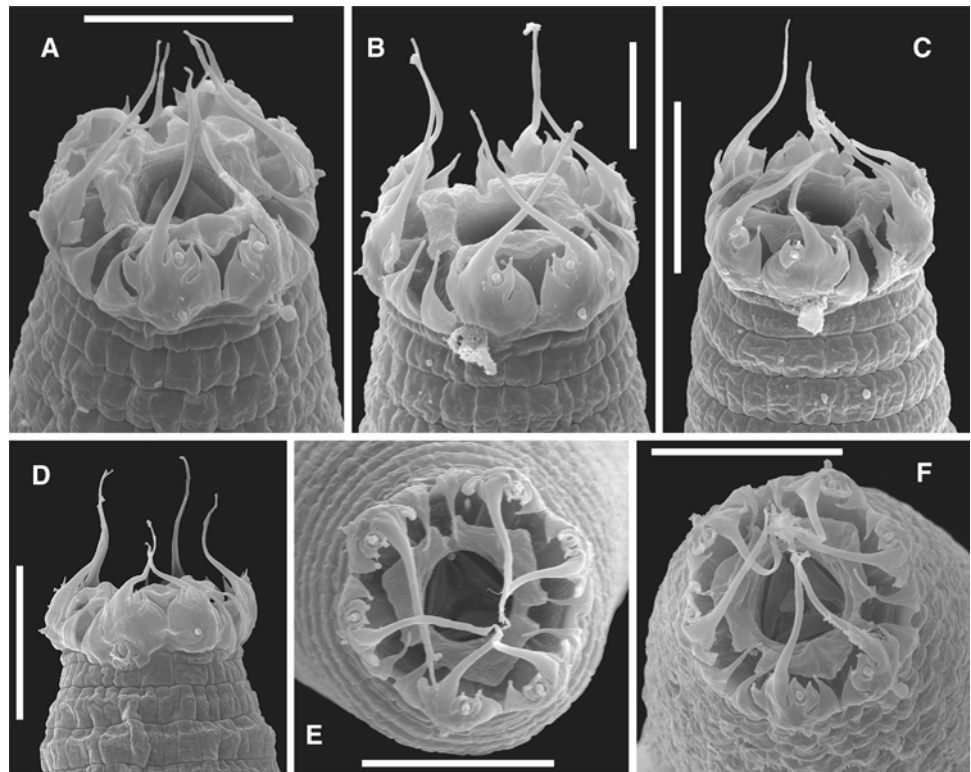
**Table 2** List of new nematode LSU ribosomal RNA gene sequences (partial) obtained in this study

Accession number	Genus/species	Locality
HM439765	<i>Cervidellus cancellatus</i>	Soil, Lviv Botanical garden, Ukraine
HM439766	<i>Cervidellus</i> cf. <i>capreolus</i>	Dune sand, Mojave desert, CA, USA
HM452377	<i>Cervidellus cervus</i>	Soil under <i>Ferocactus</i> , Sonoran desert, CA, USA
HM439767	<i>Eucephalobus mucronatus</i>	Soil, Kjettslinge, Sweden
HM439768	<i>Eucephalobus oxyuroides</i>	Soil, Kjettslinge, Sweden
HM439769	<i>Eucephalobus striatus</i>	Soil, Kjettslinge, Sweden
HM439770	<i>Eucephalobus</i> sp.	Soil, Santa Rosa Plateau Reserve, CA, USA
HM439771	<i>Panagrolobus</i> sp.	Rotting wood, James San Jacinto Mt. Reserve, CA, USA
HM439772	<i>Pseudacrobeles bostromi</i>	Rotting wood, Roztochya reserve, Ukraine
HM439773	<i>Scottnema lindsayae</i>	#24, Sand, Taylor Valley, Antarctica
HM439774	<i>Scottnema lindsayae</i>	#25, Sand, Taylor Valley, Antarctica

**Fig. 1** *Scottnema lindsayae* Timm, 1971. **a** Male tail, **b** Female tail, **c** Anterior end, surface view, **d** Pharyngeal region, **e** Female reproductive system, anterior part. Scale bar 20  $\mu$ m



**Fig. 2** *Scottnema lindsayae* Timm, 1971. **a** Anterior end, oblique view (ventral on left side down), **b** anterior end, lateral view (ventral on right side), **c** anterior end, lateral view (ventral on left side), **d** anterior end, lateral view (ventral on right side), **e** anterior end, apical view (ventral up), **f** anterior end, oblique view (ventral on right side). Scale bars 10  $\mu\text{m}$  (**a**, **c**–**f**), 5  $\mu\text{m}$  (**b**)



one short tine flanking the primary axils; one long slender process, 8–10  $\mu\text{m}$  long from lip surface, directed forward or somewhat inwards; one two-pointed (biacute) tine surrounding the labial papilla; and one triangular-pointed tine in the secondary axils. Three short, broadly triangular and flattened labial probolae, joined basally and separated by U-shaped indentations. Stoma 1.2–1.4 times as long as lip region diameter, divided into cheilostom, gymnostom and stegostom. Cheilostom wide with narrow, curved, grain-like rhabdia; gymnostom short and funnel-shaped with weakly sclerotized rhabdia; stegostom with well-developed rhabdia divided into: prostegostom constituting about half of stegostom length, and meso-, meta- and telostegostom constituting the other half of stegostom length; metastegostom with a dorsal denticle. Pharynx cephaloboid. Pharyngeal procorpus cylindrical; isthmus narrower than metacorpus, often clearly delineated from it by a break in the musculature. Basal pharyngeal bulb oval, with strongly developed valves. Cardia distinct, conoid, enveloped by intestinal tissue. Nerve ring encircling isthmus; excretory pore opens posterior to nerve ring; deirid at level of isthmus-bulb junction or at level with bulb.

#### Female

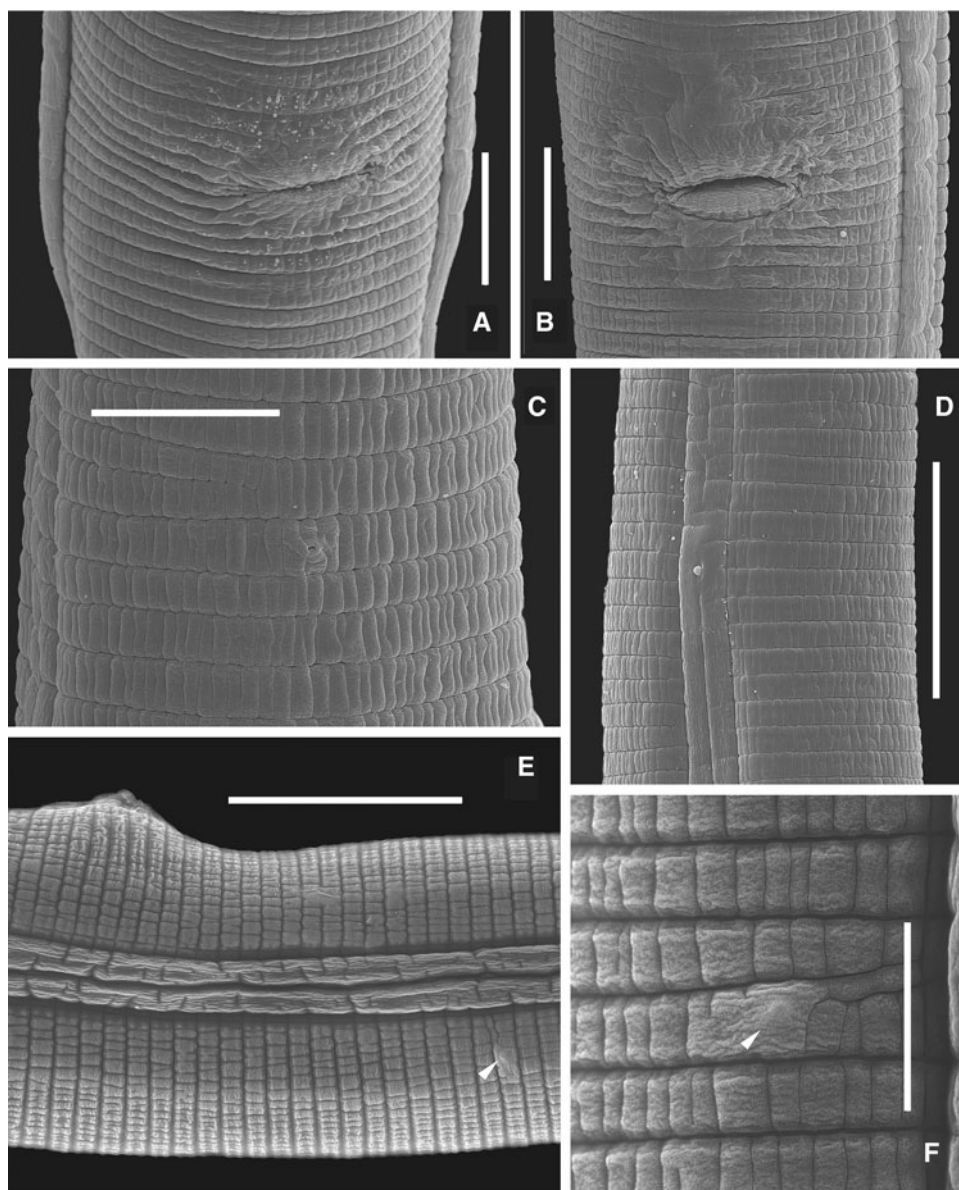
Reproductive system monodelphic, prodelphic; ovary branch antidromously reflexed, generally on right-hand

side of intestine (dextral). Part of ovary posterior to vulva straight. Spermatheca offset, directed anteriorly between oviduct and uterus, generally filled with rounded spermatozoa. Vulva a transverse slit with slightly protruding lips, vulval opening located in a somewhat left-ventrosublateral position. A pair of dorsosublateral papillae present about 1.5 body diameters posterior to vulva. Postvulval uterine sac moderately developed, generally shorter than one vulval body diameter long. Vagina straight. Rectum 1.1–1.7 times longer than anal body diameter. Phasmids 16–23  $\mu\text{m}$  posterior to anal opening, at 40–49% of tail length. Tail conoid with 19–24 ventral annuli, tapering to a pointed terminus.

#### Male

Reproductive system monorchic; testis generally on right-hand side of intestine (dextral), reflexed anteriorly, reflexed part 48–77  $\mu\text{m}$  long; a ventral reflex was observed in 60% of the studied specimens, but ventro-lateral, ventro-dorsal (“pretzel”-shaped), dorsal and dorso-lateral reflexes were also observed. Spicules cephaloboid, paired and symmetrical, curved ventrad; with narrow rounded manubrium and conoid shaft. Gubernaculum wedge-shaped. Tail conoid, arcuate ventrad, tapering to a pointed terminus, terminal part constituting a 7- to 9- $\mu\text{m}$ -long “spike”. All three lateral incisures extend posteriorly to lateral papillae at

**Fig. 3** *Scottnema lindsayae* Timm, 1971. **a–b** Vulval region, ventral view, **c** Excretory pore, **d** Lateral field and deirid, **e** Vulval region, lateral view, **f** Postvulval dorsosublateral papilla (arrowheads in **e** and **f** point at papilla). Scale bars 10  $\mu$ m (**a–c**), 20  $\mu$ m (**d, e**), 5  $\mu$ m (**f**)



midtail and the two outer incisures extend almost to tail terminus. Phasmid located 21–27  $\mu$ m posterior to cloacal opening, at 39–56% of tail length. Male genital papillae arranged as follows: three pairs of ventrosublateral precloacal papillae (one pair adcloacal, one pair at about 40–60  $\mu$ m and one pair at about 80–105  $\mu$ m anterior to cloaca); a single midventral papilla on anterior cloacal lip, two pairs at midtail (one subventral and one lateral) and three pairs at base of “spike” (one subventral, one lateral and one subdorsal).

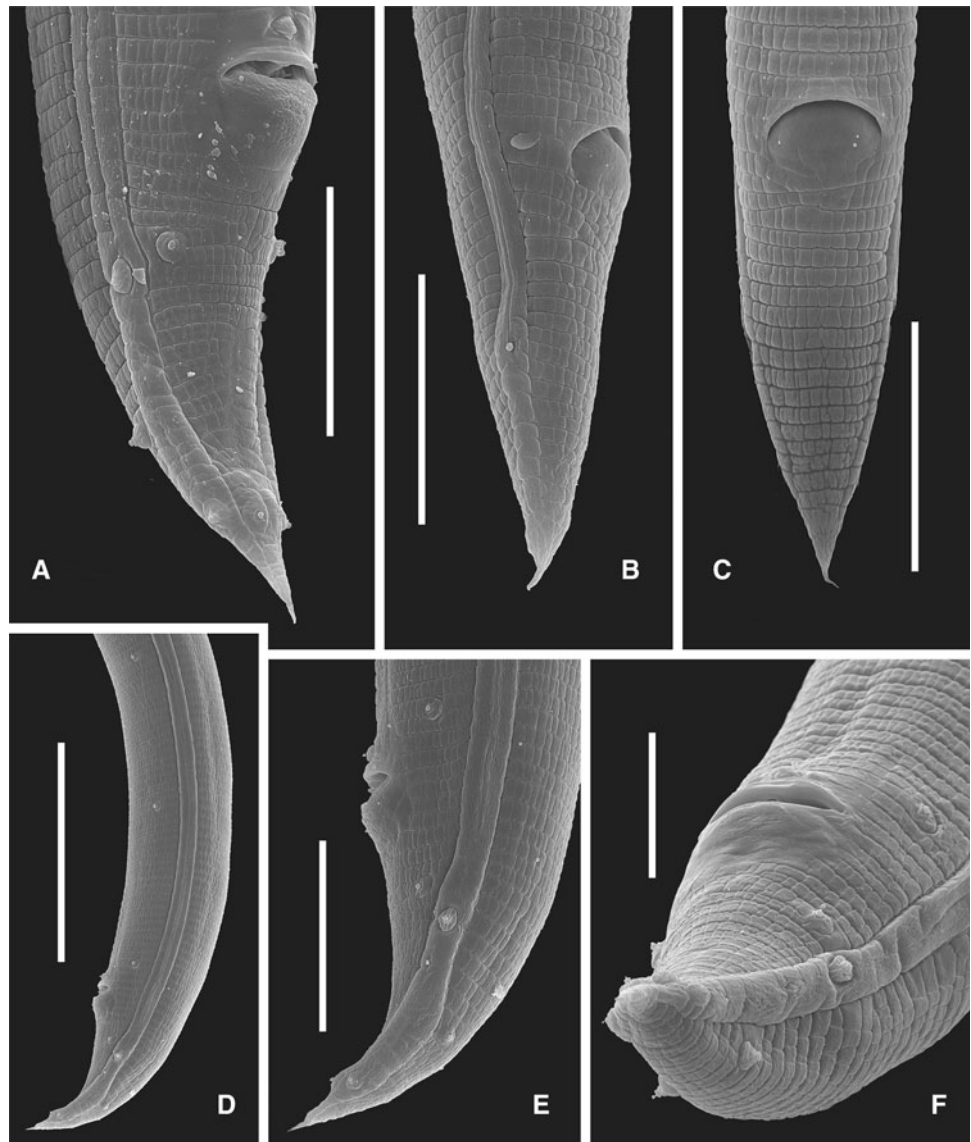
#### Taxonomic remarks

The specimens described agree well in general morphology with the original description of *S. lindsayae* by Timm

(1971) and subsequent descriptions by Vinciguerra (1994) and Andr  ssy (1998). A comparison of the ranges of some measurements and ratios among the specimens described by these authors and those described here is made in Table 4. The comparison is somewhat restricted because many measurements made in this study were not recorded in all previous descriptions and also the number of specimens measured varies. The specimens described by Timm (1971) and Vinciguerra (1994) are larger than those described by Andr  ssy (1998) and in this study ( $L = 0.72\text{--}0.86$  mm vs.  $L = 0.57\text{--}0.73$  mm for females and  $L = 0.64\text{--}0.88$  mm vs.  $0.54\text{--}0.73$  mm for males, respectively), although there is some overlap of the ranges. The total length of the specimens will consequently affect some other measurements and ratios. The somewhat



**Fig. 4** *Scottnema lindsayae* Timm, 1971. **a** Male tail, subventral view, **b** Female tail, subventral view, **c** Female tail, ventral view, **d** Male posterior end, lateral view, **e** Male tail, lateral view, **f** Male tail terminus, subdorsal view. Scale bars 20  $\mu$ m (**a–c, e**), 50  $\mu$ m (**d**), 10  $\mu$ m (**f**)



right-ventrosublaterally displaced position of vulval opening was not mentioned in any previous description and might not be evident unless specimens are studied by SEM. Timm (1971) recorded a pair of dorsosublateral papillae present somewhat posterior to vulva, but they were not mentioned by Vinciguerra (1994) or Andr  ssy (1998). Although the papillae are difficult to observe, their presence could be confirmed both by LM and by SEM (Fig. 3e–f) in this study. The number and position of male genital papillae and position of phasmid agree well with the original description (Timm 1971). Andr  ssy (1998), apart from the adcloacal pair (considered as caudal papillae), recorded two or three pairs of ventrosublateral precloacal papillae (one pair at 41–43  $\mu$ m, one pair at 75–98  $\mu$ m and one pair at 140–170  $\mu$ m anterior to cloaca), of which the most posterior pair is not always present.

#### Phylogenetic analysis

The phylogenetic relationships of the *Scottnema lindsayae* isolates were inferred from the secondary structure-based alignment of the 5' end (D1–D3) region of nuclear large subunit rDNA sequences. Bayesian inference was performed using three different substitution models that treated non-paired (“loops”) and paired (“stems”) sites differently. Two independent mcmc analyses were conducted for each of the three models. Bayesian posterior probabilities and tree topologies were produced by constructing majority-rule consensus trees after discarding trees from the burn in phase. Tree topologies from the two independent “runs” of each were identical, but clade posterior probabilities varied up to 5% between runs. All three models yielded identical results regarding the

**Table 3** Measurements (in  $\mu\text{m}$ ) of *Scottnema lindsayae* Timm, 1971 from Taylor Valley, Antarctica, presented as mean  $\pm$  SD and (range) or only range

Sample	21	22	23	24	25		27		29	
No. and sex	2 ♀♀	1 ♀	1 ♀	4 ♀♀	5 ♂♂	10 ♀♀	10 ♂♂	5 ♀♀	5 ♂♂	4 ♂♂
Body length	598–607	566	631	618 $\pm$ 35 (582–663)	612 $\pm$ 50 (539–677)	621 $\pm$ 31 (572–673)	641 $\pm$ 27 (602–691)	692 $\pm$ 31 (656–730)	672 $\pm$ 32 (618–701)	676 $\pm$ 28 (658–717)
Body diameter (BD)	37–39	33	39	34 $\pm$ 0.5 (34–35)	33 $\pm$ 3.1 (28–36)	40 $\pm$ 1.4 (37–41)	35 $\pm$ 3.3 (30–40)	41 $\pm$ 3.4 (39–47)	37 $\pm$ 2.4 (34–40)	36 $\pm$ 2.4 (34–39)
Pharynx length	148	141	153	146 $\pm$ 4.5 (139–148)	147 $\pm$ 6.3 (141–157)	148 $\pm$ 4.4 (139–153)	148 $\pm$ 7.5 (139–162)	150 $\pm$ 3.2 (146–153)	147 $\pm$ 3.3 (143–152)	148 $\pm$ 4.4 (143–153)
Tail length	40	37	43	42 $\pm$ 1.7 (40–44)	48 $\pm$ 1.3 (47–50)	43 $\pm$ 2.5 (40–47)	49 $\pm$ 3.3 (43–53)	47 $\pm$ 2.1 (46–51)	51 $\pm$ 2.8 (48–54)	50 $\pm$ 2.2 (48–53)
Anal or cloacal diam. (ABD)	18–19	17	21	19 $\pm$ 0.5 (18–19)	26 $\pm$ 0.5 (25–26)	20 $\pm$ 0.8 (19–21)	26 $\pm$ 0.9 (25–28)	20 $\pm$ 0.7 (19–21)	28 $\pm$ 1.5 (26–30)	28 $\pm$ 0.8 (29–29)
a	15.6–16.2	17.2	16.2	18.0 $\pm$ 0.8 (17.1–18.9)	18.4 $\pm$ 1.2 (17.1–19.9)	15.7 $\pm$ 0.9 (14.5–16.8)	18.3 $\pm$ 1.6 (16.3–20.9)	17.0 $\pm$ 1.6 (14.6–18.7)	18.3 $\pm$ 1.1 (17.1–20.0)	19.0 $\pm$ 1.4 (16.9–19.9)
b	4.0–4.1	4.0	4.1	4.2 $\pm$ 0.3 (3.9–4.5)	4.2 $\pm$ 0.3 (3.7–4.4)	4.2 $\pm$ 0.2 (3.9–4.4)	4.3 $\pm$ 0.2 (4.0–4.6)	4.6 $\pm$ 0.3 (4.4–5.0)	4.6 $\pm$ 0.2 (4.3–4.8)	4.6 $\pm$ 0.1 (4.4–4.7)
c	15.0–15.2	15.3	14.7	14.7 $\pm$ 0.3 (14.3–15.1)	12.8 $\pm$ 0.8 (11.5–13.5)	14.5 $\pm$ 1.0 (13.3–15.9)	13.2 $\pm$ 0.8 (11.8–14.7)	14.6 $\pm$ 0.8 (14.0–15.9)	13.3 $\pm$ 1.2 (11.4–14.0)	13.5 $\pm$ 0.4 (12.9–13.8)
c'	2.1–2.2	2.1	2.1	2.2 $\pm$ 0.1 (2.1–2.4)	1.9 $\pm$ 0.1 (1.8–2.0)	2.1 $\pm$ 0.1 (2.0–2.4)	1.8 $\pm$ 0.1 (1.7–2.0)	2.3 $\pm$ 0.1 (2.2–2.5)	1.8 $\pm$ 0.1 (1.7–2.0)	1.8 $\pm$ 0.1 (1.7–1.9)
Vulva or testis	387–388	360	404	399 $\pm$ 23 (381–431)	324 $\pm$ 39 (263–361)	402 $\pm$ 19 (372–434)	350 $\pm$ 21 (317–387)	444 $\pm$ 21 (414–468)	362 $\pm$ 34 (316–407)	362 $\pm$ 21 (343–392)
V or T %	64–65	64	64	64 $\pm$ 1.5 (63–66)	53 $\pm$ 2.9 (49–57)	65 $\pm$ 1.2 (63–67)	55 $\pm$ 2.2 (51–59)	64 $\pm$ 0.8 (63–65)	54 $\pm$ 4.1 (50–59)	54 $\pm$ 1.7 (51–55)
Vulva-anus/tail	4.3–4.4	4.5	4.3	4.2 $\pm$ 0.2 (3.9–4.5)	–	4.1 $\pm$ 0.4 (3.7–4.8)	–	4.3 $\pm$ 0.2 (4.1–4.7)	–	–
Lip region diameter	18–19	17	18	17–18	17–18	17–19	16–18	18–20	17–18	17–18
Stoma length	22–23	22	23	21–23	21–23	21–24	21–23	23–24	21–23	22–24
Corpus length	64–65	63	66	63–68	62–67	63–69	62–70	63–67	60–67	61–67
Isthmus length	34	32	37	34–36	32–41	31–37	31–40	35–37	33–36	36–37
Bulb length	28–30	27	31	27–30	26–31	27–31	28–32	29–31	28–32	27–28
Bulb diameter	22–24	22	23	21–24	21–24	21–25	20–24	23–27	22–29	21–22
Corpus/isthmus ratio	1.9	2.0	1.8	1.8–2.0	1.6–2.0	1.8–2.1	1.7–2.1	1.8–1.9	1.7–2.0	1.7–1.8
Nerve ring from ant. end	96–106	94	116	101–117	102–110	102–122	99–121	114–129	108–118	106–122
Excr. pore from ant. end	106–121	105	123	111–133	112–120	117–135	112–132	123–143	122–134	118–137
Deirid from ant. end	119–135	122	141	128–146	131–136	131–151	128–148	131–159	140–160	137–153
R <sub>NR</sub>	27–30	27	32	29–32	27–31	28–31	26–34	29–33	27–31	26–30
R <sub>EP</sub>	30–35	32	35	32–37	31–36	32–36	31–38	32–37	31–35	30–35



**Table 3** continued

Sample	21	22	23	24	25	27	29
No. and sex	2 ♀♀	1 ♀	1 ♀	4 ♀♀	10 ♀♀	5 ♀♀	5 ♀♀
R <sub>DEI</sub>	34–40	40	42	38–42	38–41	35–42	38–40
Testis flexure length	–	–	–	–	–	–	–
Spermatheca or spicule length	36–44	23	39	38 ± 5.2 (33–45)	39 ± 2.4 (34–42)	49 ± 7.8 (39–58)	46 ± 3.8 (42–51)
PUS or gubernaculum length	24–26	24	27	23 ± 8.6 (13–34)	22 ± 7.5 (11–30)	26 ± 10.8 (10–34)	32 ± 2.4 (29–34)
PUS/VBD	0.7–0.8	0.8	0.8	0.4–1.1	0.3–0.9	0.3–1.0	0.7–0.9
Rectum	25	25	29	24–25	23–31	25–28	28–29
Rectum/ABD	1.3	1.5	1.4	1.3–1.4	1.1–1.7	1.2–1.4	1.3–1.4
Phasmid	18	16	19	20 ± 1.2 (19–21)	20 ± 1.8 (18–23)	20.5 ± 1.0 (20–22)	19–20 (n = 3)
Phasmid (% of tail)	45	45	44	47 ± 1.7 (45–48)	48 ± 1.9 (45–49)	43.5 ± 2.9 (40–47)	41–44 (51–52)

<sup>a</sup> Number of annuli from anterior end to nerve ring, excretory pore and deirid, respectively

**Table 4** Comparison of measurements (in  $\mu\text{m}$ ) of *Scottnema lindsayae* Timm, 1971 from Antarctica

Author	Timm (1971)		Vinciguerra (1994)		Andrássy (1998)		Present populations	
No. and sex	20 ♀♀	20 ♂♂	2 ♀♀	5 ♂♂	8 ♀♀	6 ♂♂	28 ♀♀	24 ♂♂
Body length (mm)	0.74–0.86	0.71–0.88	0.72–0.78	0.64–0.79	0.64–0.72	0.55–0.73	0.57–0.73	0.54–0.72
Body diameter	–	–	–	–	34–43	28–36	33–47	28–40
Pharynx length	–	–	144–181 <sup>a</sup>	144–181 <sup>a</sup>	125–170	–	139–153	139–162
Tail length	–	–	49–51	49–59	41–48	41–48	37–51	43–54
a	15–22	16–22	20–21	17–24	16–20	19–22	14–19	16–21
b	4.2–4.8	4.2–4.8	4.3–4.5	4.1–5.0	3.8–4.6	4.1–4.3	3.9–5.0	3.7–4.8
c	13–18	13–17	14–16	11–15	14–16	13–15	13–16	11–15
c'	1.9–2.8	1.4–2.1	2.4–2.5	1.7–2.0	2.4–2.7	2.0–2.2	2.0–2.5	1.7–2.0
V or T %	57–66	52	64–65	33–39	61–65	–	62–67	49–59
Vulva-anus/tail	–	–	–	–	3.9–4.7	–	3.7–4.8	–
Spermatheca or spicules	–	35–39	–	33–35	–	30–37	23–58	30–38
PUS or gubernaculum	Present	17	42–45	16	Present	18–22	10–34	15–18
PUS/VBD	–	–	–	–	≤1	–	0.3–1.1	–

<sup>a</sup> Only one range given for adults



**Fig. 5** Phylogenetic position of *Scottinema lindsayae* Timm, 1971 within the family Cephalobidae. Majority-rule consensus tree of the Bayesian phylogenetic analysis (RNA16A model) of Cephalobomorpha LSU rDNA (D1–D3 region). Tree rooted using *Geraldus* sp. as the outgroup; branch lengths represent the mean posterior estimates of

the expected number of substitutions per site. Bayesian posterior probabilities (BI) and bootstrap support (ML) are shown for each branch in the following order: RNA16A/RNA7A/RNA7D/ML bootstrap. Clades that were resolved in ML bootstrap analysis (bootstrap of 50% or higher) are also shown in **bold** (branches) in the tree

sister-taxon relationship of *S. lindsayae*; the Bayesian consensus tree based on the RNA16A model showed slightly greater overall resolution and was therefore selected for presentation (Fig. 5) and discussion. Bayesian consensus tree topologies were identical for the RNA7A and RNA7D models, whereas the topology recovered for the RNA16A model was different with respect to the position of two species. First, with the RNA16A model, *Penjatinema novaezeelandiae* Holovachov, Boström, Tandingan De Ley, Nadler & De Ley, 2009 was the sister taxon to the clade composed of *Cervidellus* sp. JB138 and *Nothacrobeles borregi* Poiras, Baldwin, Mundo-Ocampo & Bumbarger, 2002 (PP 67%). In contrast, when using the RNA7A and RNA7D models, this part of the phylogeny

was unresolved. The second difference involved the position of *Placodira lobata* Thorne, 1937. With the RNA16A model, *P. lobata* was the sister taxon to a clade composed of *Zeldia spannata* Waceke, Bumbarger, Mundo-Ocampo, Subbotin & Baldwin, 2005 and *Chiloplacus* sp. JB81 (PP 50%). In contrast, for the RNA7A and RNA7D models, this part of the phylogeny was unresolved. For all three substitution models, Bayesian analyses recovered *S. lindsayae* as a monophyletic sister group to the genus *Stegelletina* Andrassy, 1984 with higher posterior probability; maximum likelihood analysis yielded the same result with high bootstrap support (Fig. 5). This clade of *Scottinema* plus *Stegelletina* is nested within the “grade” of taxa with complex labial morphology. A separate maximum

likelihood (ML) analysis of the same alignment, but without stem-loop partitioning was implemented using RAxML (Stamatakis et al. 2008). The resulting ML bootstrap tree showed low support for many clades, including several clades that received moderate to high posterior probabilities in the Bayesian analyses. Although there was topological agreement between certain clades in the ML bootstrap and Bayesian analyses (Fig. 5), many other nodes in the Bayesian tree were not resolved in the ML bootstrap (50% majority-rule) consensus tree. By comparison, using a partitioned dataset and base-paired substitution models in a Bayesian context provided greater resolution, although Bayesian posterior probabilities and bootstrap values are not directly comparable (Alfaro et al. 2003). In addition, using a more complex sixteen-state model (RNA16A) produced only a slightly different phylogenetic hypothesis compared to less complex seven-state models (RNA7A and RNA7D). The major difference between seven-state and sixteen-state models is how they “treat” non-complementary pairs (“mismatches”) in stem regions of RNA. We conclude (and visual inspection of the alignment confirms) that the number of “mismatches” in paired sites (“stems”) is too small for these different models to differentially affect the phylogenetic analysis. Previous studies showed that neither using more of the LSU rDNA gene (Nadler et al. 2006; Smythe and Nadler 2007), or using more taxa but shorter LSU rDNA sequences (Holovachov et al. 2010) are sufficient to satisfactorily resolve the phylogenetic relationships within Cephalobidae, and additional phylogenetically informative genes will be required to achieve this goal.

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